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Prevention of browning of depolymerized chitosan obtained by gamma irradiation

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ABSTRACT

In this paper, effect of oxygen and pH on the browning of chitosan exposed to gamma radiation was investigated. It was found that oxygen and pH value could play important roles in the inhibiting browning of irradiated chitosan. When the pH value of chitosan solution was below 3.0, sufficient oxygen could inhibit browning of irradiated chitosan in aqueous solution. As a result of irradiation of chitosan solution (pH < 3) with sufficient oxygen, the irradiated chitosan solutions obtained were colorless and pellucid. FT-IR, ^{13}C NMR, and UV–vis spectra confirmed that the irradiation in the presence of oxygen cannot result in chemical modification of irradiated chitosan. An effective technology was developed for the inhibition of browning of irradiated chitosan during depolymerization of chitosan by gamma irradiation.

1. Introduction

Chitosan, the linear and typically 20% acetylated (1-4)-2-amino-2-deoxy-β-D-glucan, is isolated industrially from marine chitin. Owing to its biodegradability, biocompatibility, non-toxicity and versatile physico-chemical properties, chitosan has a great potential for a wide range of functions and applications in many fields (Muzzarelli, Boudrant, et al., 2012), such as food, pharmaceutics (Zhou, Zhang, Zhang, & Chen, 2011), biomaterials (Li, Wang, Wu, Zhang, & Hu, 2010; Sashiwa & Aiba, 2004; Shi & Tan, 2002; Zhao, Li, Li, Zhou, & Li, 2011), tissue engineering (Muzzarelli, Greco, et al., 2012; Tang et al., 2010; Zhang et al., 2010), and environmental protection (Muzzarelli, 2011; Muzzarelli & Tubertini, 1972). Chitosan and its derivatives have exhibited high antimicrobial activity against a wide variety of pathogenic and spoilage microorganisms (Guo, Ren, Dong, Wang, & Li, 2013; Hu et al., 2007; Kong, Chen, Xing, & Park, 2010).

However, these functions and applications have been revealed to be dependent not only upon the chemical structure of chitosan but also its molecular size (Xia, Liu, Zhang, & Chen, 2011; Xu, Zhao, Han, & Du, 2007). For example, it has been reported that low-molecular-weight chitosan and chitooligosaccharides demonstrate various kinds of novel biological activities (Dong et al., 2004; Dou, Ma, Xiong, Tan, & Du, 2011; Je, Park, & Kim, 2004; Kim and Rajapakse, 2005; Wu, Yao, Bai, Du, & Ma, 2010; Xu et al., 2008; Yin, Zhao, & Du, 2010).

To convert the high-molecular-weight chitosan into the low-molecular-weight chitosan or chitooligosaccharides, gamma irradiation has been used for the depolymerization of chitosan (Choi, Ahn, Lee, Byun, & Park, 2002; Feng, Du, Li, Hu, & Kennedy, 2008; Hai, Diep, Nagasawa, Yoshii, & Kume, 2003; Kang, Dai, Zhang, & Chen, 2007; Matsuhashi & Kume, 1997; Ulański & Rosiak, 1992; Wasikiewicz, Yoshii, Nagasawa, Wach, & Mitomo, 2005; Yoksan, Akashi, Miyata, & Chirachanchai, 2004). Unfortunately, the depolymerization induced by gamma irradiation was also accompanied simultaneously by the browning of irradiated chitosan according to Choi et al. (2002) and Yoksan et al. (2004). It was also found that gamma irradiation could lead to browning of the vacuum-packed chitosan rod with high bending strength and potential application as internal fixation of bone fracture (Shen, Hu, Wang, & Qu, 2011). The browning resulted from the gamma irradiation is a vital disadvantage, which can influence the properties of the low-molecular-weight chitosan. It has been reported that the browning has certain impacts on the properties of chitooligomers (Zeng et al., 2007). For example, the solubility of chitooligomers decreased when the sample was severely browning and that some black sample was insoluble even in strong acidic condition. It was also found that the thermal stability and moisture-adsorption of chitooligomers decreased with the increase of browning.

In addition to the depolymerization of chitosan mentioned above, irradiation of chitosan or chitin for other purposes and

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applications were also studied by researchers (Casimiro, Gil, & Leal, 2010; Duy, Phu, Anh, & Hien, 2011; Hien, Phu, Duy, & Lan, 2012; Ocloo et al., 2011; Pasanphan, Rimdusit, Choofong, Piroonpan, & Nilsuwankosit, 2010; Rahman et al., 2013; Singh & Singh, 2012). Those authors did not mention browning, or the reasons for the discoloration in their articles because those research topics might be irrelevant to the browning of irradiated chitosan.

Although gamma irradation of chitosan was investigated by many researchers, the effect of oxygen and pH on the browning of chitosan exposed to gamma radiation has not yet been reported.

The aim of the present work was to investigated the effect of oxygen and pH on the browning of chitosan exposed to gamma radiation. A chemical technology for inhibition of browning of irradiated chitosan was proposed. With the proposed chemical technology, the irradiation-induced depolymerization of chitosan will not be accompanied by the browning of irradiated chitosan.

2. Materials and methods

2.1. Materials

The commercial chitosan A (Purity 92.73%; Mw 440.20 kDa, Mw/Mn 1.95; DD 91.20%); The commercial chitosan B (Purity 90.27%; Mw 490.59 kDa, Mw/Mn 1.72; DD 90.77%) originating from shrimp shells. They were all purchased from Jinke Biochemical Ltd. (Zhejiang, China). The chitosan C (Purity 89.22%; Mw 410.73 kDa, Mw/Mn 1.66; DD 91.10%), which originated from crab shells, was prepared in laboratory. Mw, Mw/Mn and DD of the chitosan samples used in this study were determined experimentally. In order to exempt the samples from inorganics and pigments, the commercial chitosan B and the chitosan C were further purified in laboratory. The purity of the chitosan samples were calculated after their purification.

Gaseous oxygen purity was above 99%. Gaseous nitrogen purity was above 99%. All other chemicals were of reagent grade. 2-l reaction vessels (i.e. 2000 ml polyethylene terephthalate (PET) vessels) were used in this experiment.

A ^{60}Co source, which was employed for gamma irradiation of chitosan, was set up in Zibo Liyuan Hi-Tech Irradiation Technology Ltd. (Zibo, Shandong, China).

2.2. Characterization

FT-IR spectra were recorded in powder form in KBr discs in the range of 4000–400 cm^{-1} on a Nicolet 5DXB FT-IR spectrophotometer. Thirty-two scans at a resolution of 4 cm^{-1} were averaged and referenced against air.

^{13}C NMR spectra were recorded on BRUKER AVANCE-500NMR SPECTROMETER. The chitosan samples were converted into their hydrochloride salts and then were dissolved in D_2O.

UV–vis absorption spectra were obtained using a UV–vis spectrophotometer Agilent 8453 in the range of 200–400 nm.

Inhibitory effect on browning of irradiated chitosan during depolymerization of reference chitosan by gamma irradiation was determined by measuring $Abs_{267Ir} - Abs_{267Re}$. Abs_{267Ir} is the absorbance at 267 nm of irradiated chitosan solution, and Abs_{267Re} is the absorbance at 267 nm of reference chitosan solution.

Weight-average molecular weight (Mw), number-average molecular weight (Mn) and molecular weight dispersion (Mw/Mn) of chitosan samples were measured by gel permeation chromatography (GPC). The GPC equipment consisted of connected columns (TSK G5000-PW and TSK G3000-PW), TSP P100 pump and RI 150 refractive index detector. The similar method was used according to Feng et al. (2008).

The degree of deacetylation (DD) was determined using the method reported in literature (Tolaimate et al., 2000). The chitosan sample (0.1 g) was completely dissolved in a known excess of 0.1 M HCl solution (10 ml). From the titration of this solution with a 0.1 M NaOH solution, a curve with two inflection points was obtained. The amount of acid consumed between these two points was considered to correspond to the amount of amino groups of chitosan.

Intrinsic viscosity was determined using an Ubbelohde capillary viscometer (inner diameter 0.5 mm). The running times of the solutions were measured in triplicate in a constant-temperature water bath at $25 \pm 0.5\,^{\circ}C$.

2.3. Adjusting oxygen partial pressure for inhibition of browning during depolymerization of chitosan by gamma irradiation

The reference chitosan solutions (1%, w/v, pH 2.0, and 400 ml) were prepared by dissolving the chitosan sample (commercial chitosan A, purified chitosan B and C) in the dilute hydrochloric acid solution and then introduced in the 2-l PET vessels. The vessels containing chitosan solution were filled with oxygen (201.325 kPa, 25 °C, 1600 ml), oxygen (151.325 kPa, 25 °C, 1600 ml), oxygen (101.325 kPa, 25 °C, 1600 ml), air (101.325 kPa, 25 °C, 1600 ml), and nitrogen (101.325 kPa, 25 °C, 1600 ml), respectively. Thus, oxygen partial pressures in the vessels were kept at 201.325, 151.325, 101.325, 21.227, and 0 kPa, respectively. The chitosan solutions under different partial pressure of oxygen were irradiated with doses of 25 kGy in the irradiation room. The irradiated chitosan solutions were obtained after the irradiation. The absorbance of the irradiated chitosan solutions was measured at 267 nm by UV–vis spectrophotometer. Inhibitory effect on browning of irradiated chitosan was determined by calculating $Abs_{267Ir} - Abs_{267Re}$.

2.4. Adjusting pH value for inhibition of browning during depolymerization of chitosan by gamma irradiation

The reference chitosan solutions (1%, w/v, 400 ml) with pH 1.0, 2.0, 3.0, 4.0, and 5.0 were prepared by dissolving the chitosan sample (commercial chitosan A, purified chitosan B and C) in the dilute hydrochloric acid solution and then introduced in the 2-l PET vessels, respectively. The PET vessels containing reference chitosan solution were filled with 1600 ml oxygen (25 °C, 101.325 kPa) and sealed, respectively. The chitosan solutions (1%, w/v, 400 ml, with different pH values) under oxygen partial pressure of 101.325 kPa were irradiated with doses of 25 kGy. Inhibitory effect on browning of irradiated chitosan was determined by calculating $Abs_{267Ir} - Abs_{267Re}$.

2.5. Irradiation of freeze-dried chitosan samples

The reference chitosan solutions (1%, w/v, 400 ml) with pH 1.0, 2.0, 3.0, 4.0, and 5.0 were prepared by dissolving the chitosan sample in the dilute hydrochloric acid solution. The chitosan solutions were firstly frozen and then lyophilized in a freeze dry system. Finally, the freeze-dried chitosan samples were obtained from solutions with pH 1.0, 2.0, 3.0, 4.0, and 5.0. The freeze-dried samples were put in the 2-l PET vessels, respectively. The PET vessels containing freeze-dried chitosan samples were then filled with oxygen (25 °C, 101.325 kPa, and approximately 2000 ml) and sealed, respectively. The freeze-dried chitosan samples under oxygen partial pressure of 101.325 kPa were irradiated with doses of 25 kGy in the irradiation room.

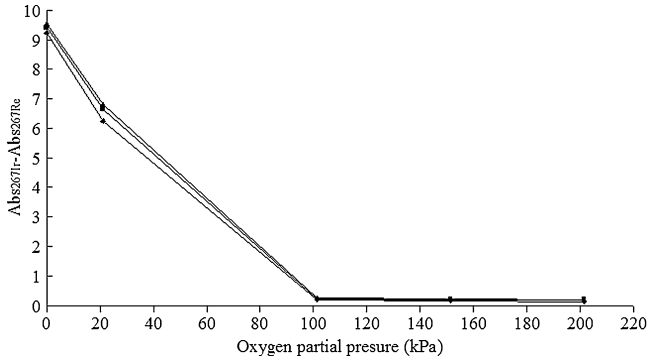

Fig. 1. Inhibitory effect of oxygen partial pressure on browning. The samples were irradiated with the 25 kGy at 25 °C. (♦) Commercial chitosan A; (■) purified chitosan B; (▲) purified chitosan C. To measure Abs_{267Ir} accurately, 1 ml of 1% (w/v) irradiated chitosan solution was diluted to 5 ml of 0.2% (w/v) solution after the irradiation in the presence of nitrogen of 101.325 kPa. Thus, $Abs_{267Ir} = 5Abs_{267Ir\ [0.2\%]}$. $Abs_{267Ir\ [0.2\%]}$ is the absorbance at 267 nm of 0.2% (w/v) irradiated chitosan solution. For the irradiation in the presence of air of 101.325 kPa, 1 ml of 1% (w/v) irradiated chitosan solution was diluted to 3 ml of solution.

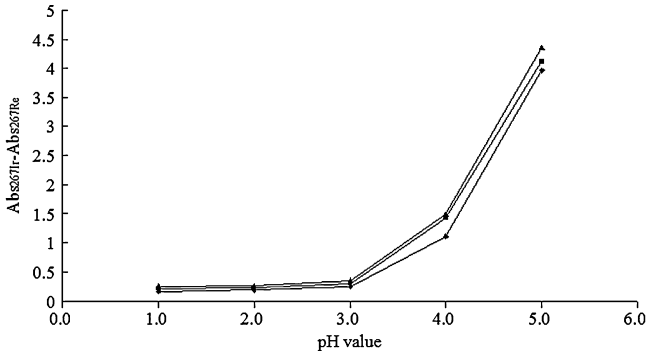

Fig. 2. Inhibitory effect of pH value on browning. The samples were irradiated under oxygen with the 25 kGy at 25 °C. (♦) Commercial chitosan A; (■) purified chitosan B; (▲) purified chitosan C. For the chitosan solution with pH 5.0, 1 ml of 1% (w/v) irradiated chitosan solution was diluted to 3 ml of solution after the irradiation.

3. Results and discussions

3.1. Inhibitory effect of oxygen partial pressure on browning during depolymerization of chitosan by gamma irradiation

In our present work, it was found that the chitosan solution which underwent gamma irradiation normally had a maximum absorption around 267 nm in UV–vis spectra. It is consistent with the previous results, where the maximum absorption peak between 250 and 280 nm was observed after depolymerization of chitosan by gamma irradiation according to Muzzarelli and Tubertini (1972) and Wasikiewicz et al. (2005). The absorbance at about 267 nm is strongly dependent upon the irradiation-induced browning of irradiated chitosan solution. The absorbance increased with increasing browning of irradiated chitosan solution. Thus, inhibitory effect on browning can be evaluated by calculating $Abs_{267Ir} - Abs_{267Re}$.

Fig. 1 shows the inhibitory effect of oxygen partial pressure on browning of irradiated chitosan during irradiation processing. It was obvious that the inhibitory effect on browning is strongly dependent upon oxygen partial pressure when the pH value of chitosan solution was kept at 2.0. The $Abs_{267Ir} - Abs_{267Re}$ was close to zero when oxygen partial pressure ranged from 101.325 to 201.325 kPa. The irradiated chitosan solutions were colorless and pellucid when oxygen partial pressure ranged from 101.325 to 201.325 kPa. However, the browning can be observed with decreasing oxygen partial pressure from 101.325 to 0 kPa. As can be seen in Fig. 1, the $Abs_{267Ir} - Abs_{267Re}$ also increased sharply with decreasing oxygen partial pressure from 101.325 to 0 kPa.

3.2. Inhibitory effect of pH value on browning during depolymerization of chitosan by gamma irradiation

Fig. 2 shows the inhibitory effect of pH value on browning of irradiated chitosan during depolymerization of chitosan by gamma irradiation. It was obvious that the inhibitory effect on browning is strongly dependent upon pH value when the oxygen pressure was kept at 101.325 kPa. The irradiated chitosan solutions were colorless and pellucid when pH value ranged from 1.0 to 3.0 during depolymerization of chitosan by gamma irradiation. The $Abs_{267Ir} - Abs_{267Re}$ was close to zero when pH value ranged from 1.0 to 3.0. However, the browning can be observed with increasing pH value from 4.0 to 5.0. The $Abs_{267Ir} - Abs_{267Re}$ also increased sharply when pH value ranged from 4.0 to 5.0.

It has been reported that $(GlcN)_1$, $(GlcN)_2$, $(GlcN)_3$, $(GlcN)_4$, $(GlcN)_5$, and $(GlcN)_6$ can be produced by the radiation degradation of chitosan according to Choi et al. (2002). The D-glucosamine oligosaccharides from monomer to hexamer are reducing sugars. The reaction between reducing sugar and $-NH_2$ (nucleophile) in chitooligomers can result in formation of melanoidins according to Zeng et al. (2007), which is brown pigmented product. However, the $-NH_2$ (nucleophile) in lower pH solution is almost converted into the $-NH_3^+$ (electrophile). The reducing sugar did not react with the $-NH_3^+$. Thus, the amount of melanoidins was significantly decreased. There was only a small amount of melanoidins generated in lower pH solution because of the ammonium dissociation. Furthermore, the irradiation under oxygen might result in the production of ozone (Nagasawa, Mitomo, Yoshii, & Kume, 2000). The ozone would lead to the oxygenolysis of the small amount of melanoidins. To confirm the above presumption, ozone was bubbled into the irradiated chitosan solution which had been brown. The brownish color of the irradiated chitosan solution and the peak at 267 nm in UV spectra almost disappeared after ozone treatment for 80 min. Thus, lower pH and oxygen would help reduce browning.

It has been reported that Maillard reaction products can be formed by irradiation of chitosan-glucose solution (Rao, Chawla, Chander, & Sharma, 2011). Glucose is a reducing sugar with reducing end in aqueous solution. $(GlcN)_1$, $(GlcN)_2$, $(GlcN)_3$, $(GlcN)_4$, $(GlcN)_5$, and $(GlcN)_6$ produced by the radiation degradation of chitosan are all reducing sugar with reducing end. The reducing sugars and free amino groups (nucleophile) can react with each other, which is commonly referred as Maillard reaction. It was found that the higher pH favors occurrence of Maillard reaction because of the increased reactivity of the amine groups by the deprotonation in higher pH solution (Hodge, 1953). In lower pH solution, however, the reactivity of the $-NH_2$ (nucleophile) was decreased when it is converted into the $-NH_3^+$ (electrophile). Furthermore, the protonated amino groups might also lead to the increased steric hindrance effect for Maillard reaction. It has been reported that the lower pH value consequently cause a slowing down of the Maillard reaction (non-enzymatic browning reaction) (Martins, Jongen, & Boekel, 2000; Oh et al., 2006). It has also been found that Maillard reactions of simpler carbohydrates proceed efficiently in the absence of oxygen (Katsuno, Shimamura, Kashiwagi, Izawa, & Ukeda, 2013; Litchfield, Thorpe, & Baynes, 1999).

In addition to oxygen partial pressure and pH value, it was also found that many factors such as storage temperature, irradiation dose, storage time, post-irradiation effect, etc. all have some influences on the value of $Abs_{267Ir} - Abs_{267Re}$.

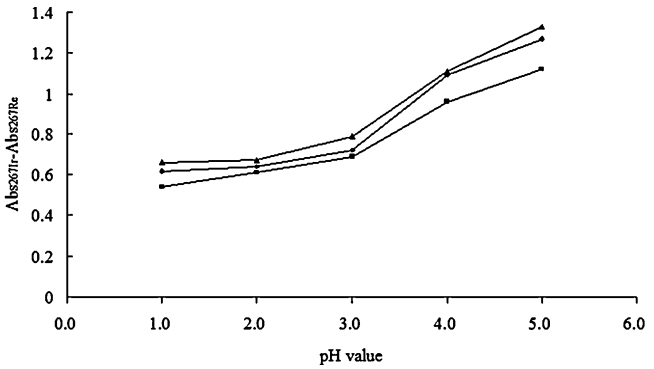

Fig. 3. $Abs_{267Ir} - Abs_{267Re}$ of the freeze-dried chitosans irradiated under oxygen. The samples were irradiated under oxygen with the 25 kGy at 25 °C. The concentration of each sample solution was 2.2% (w/v). (♦) Commercial chitosan A; (■) purified chitosan B; (▲) purified chitosan C.

Table 1
Evaluation of the chromatographic measurements of chitosan irradiated under oxygen with the 25 kGy at 25 °C.

pH	Freeze-dried chitosan A, B, C[a]		Chitosan A, B, C[a] in aqueous solution	
	Mw (kDa)	Mw/Mn	Mw (kDa)	Mw/Mn
1.0	347, 377, 332	2.44, 2.53, 2.40	12.22, 18.19, 11.78	1.37, 1.44, 1.21
2.0	335, 358, 327	2.45, 2.51, 2.42	12.97, 17.97, 11.76	1.38, 1.43, 1.19
3.0	331, 355, 322	2.57, 2.52, 2.47	11.04, 13.93, 11.70	1.35, 1.41, 1.20
4.0	327, 341, 312	2.63, 2.69, 2.57	10.17, 12.38, 11.16	1.19, 1.37, 1.19
5.0	321, 330, 304	2.66, 2.76, 2.64	11.66, 12.33, 10.85	1.30, 1.35, 1.17

[a] The samples were irradiated under oxygen (101.325 kPa) with the 25 kGy at 25 °C.

3.3. $Abs_{267Ir} - Abs_{267Re}$ of the freeze-dried chitosans

It absolutely necessary to examine a simpler system deprived of water. It is known that it is very dangerous to irradiate chitosan solutions, thus samples for sterilization or else are currently more safely irradiated as xerogels (freeze-dried materials). $Abs_{267Ir} - Abs_{267Re}$ of the freeze-dried chitosans irradiated under oxygen is shown in Fig. 3.

Although the freeze-dried samples obtained from solutions at known pH were irradiated under oxygen, the samples underwent browning. $Abs_{267Ir} - Abs_{267Re}$ increased with increasing pH value. Muzzarelli and Tubertini (1972) reported in their original work that chitosan powder suspended in acidic aqueous media at various pH values could tolerate gamma irradiation without losing its identity. It was found that the freeze-dried chitosan samples were also quite indifferent to radiation when they were irradiated in the form of solids. As can be seen in Table 1 and Fig. 4, only a small fraction of chitosan could undergo depolymerization. This might result in production of a small amount of reducing end of irradiated chitosan. Thus, there was only a very small amount of melanoidins generated in irradiated chitosan. It was found that the moisture absorption of the freeze-dried samples before and after irradiation under oxygen increased progressively with increasing pH value. The pH-dependent moisture absorption of the samples could promote the browning reaction. Furthermore, enhancement of the browning reaction was obviously observed when the freeze-dried samples irradiated under oxygen were dissolved in aqueous solution for the Abs_{267Ir} measurements. Abs_{267Ir} increased with increasing storage time and temperature. Existence of post-irradiation effect could also lead to an increase in Abs_{267Ir}.

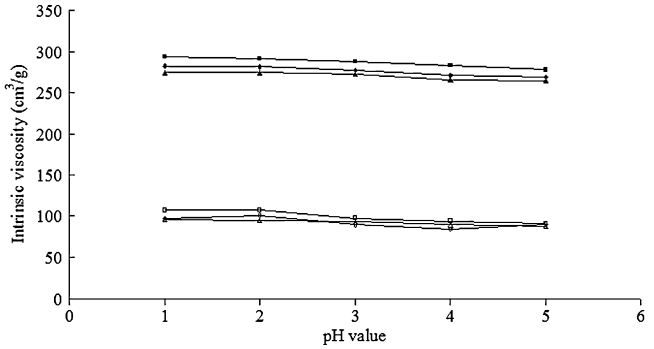

Fig. 4. The intrinsic viscosity of irradiated chitosan. The samples were irradiated under oxygen with the 25 kGy at 25 °C. (♦) Freeze-dried chitosan A; (■) freeze-dried chitosan B; (▲) freeze-dried chitosan C; (◊) chitosan A in aqueous solution; (□) chitosan B in aqueous solution; (△) chitosan C in aqueous solution.

As mentioned above, the irradiation under oxygen could result in the production of ozone according to Nagasawa et al. (2000). And the ozone could result in decolorization of the irradiated chitosan solution which had been brown. In order to clarify whether the ozone could decolorize the irradiated chitosan in solid state, the freeze-dried samples irradiated were left in ozone for 12 h. It was found that $Abs_{267Ir} - Abs_{267Re}$ did not appreciably decrease, thus the generated ozone could not decolorize the freeze-dried samples irradiated under oxygen.

3.4. Chromatographic measurements and intrinsic viscosity

The evaluation of the chromatographic measurements of irradiated chitosan is shown in Table 1. The results suggest that chitosans in aqueous solution were preferentially depolymerized and that the freeze-dried chitosan samples as xerogels were not preferentially depolymerized. It was also found that the irradiation of chitosan in queous solution resulted in the lower Mw/Mn compared with that of freeze-dried chitosan samples. Mw and Mw/Mn were slightly affected by the pH value. Existence of post-irradiation effect was found for the Mw and Mw/Mn of the irradiated chitosan samples.

The intrinsic viscosity of irradiated chitosan is shown in Fig. 4. It was found that the irradiation of chitosan in queous solution resulted in the lower intrinsic viscosity compared with that of freeze-dried chitosan samples. The intrinsic viscosity of irradiated chitosan was slightly affected by the pH value. Existence of post-irradiation effect was also found for the intrinsic viscosity of the irradiated chitosan samples.

For chitosan dissolved in the hydrochloric acid solution, the inter- and intra-molecular hydrogen bond networks were obstructed by protonation. Expansion of the chitosan chains could lead to the high possibility of depolymerization by gamma irradiation. Free radicals such as $^{\bullet}H$, $^{\bullet}HO$, $^{\bullet}HO_2$ etc., were generated due to the presence of both O_2 and H_2O in the irradiation reaction system according to Pasanphan et al. (2010). As a result, the free radicals could lead to significantly enhanced depolymerization of chitosan dissolved in the aqueous solution.

In contrast, for the freeze-dried chitosan samples, the polymer chains were not able to adequately expand due to the formation of the inter- and intra-molecular hydrogen bond networks. The chitosan samples were quite indifferent to radiation, provided that they were irradiated in the form of solids according to Muzzarelli and Tubertini (1972). Furthermore, the amount of free radicals might be significantly decreased by the absence of water when the freeze-dried samples were only irradiated under oxygen.

3.5. Effect of the irradiation in the presence of oxygen on degree of deacetylation (DD) of irradiated chitosan

To investigate the effect of irradiation in the presence of oxygen on DD of irradiated chitosan, the reference chitosan solutions (1%, w/v, 400 ml, and pH value from 1.0 to 5.0) were irradiated under

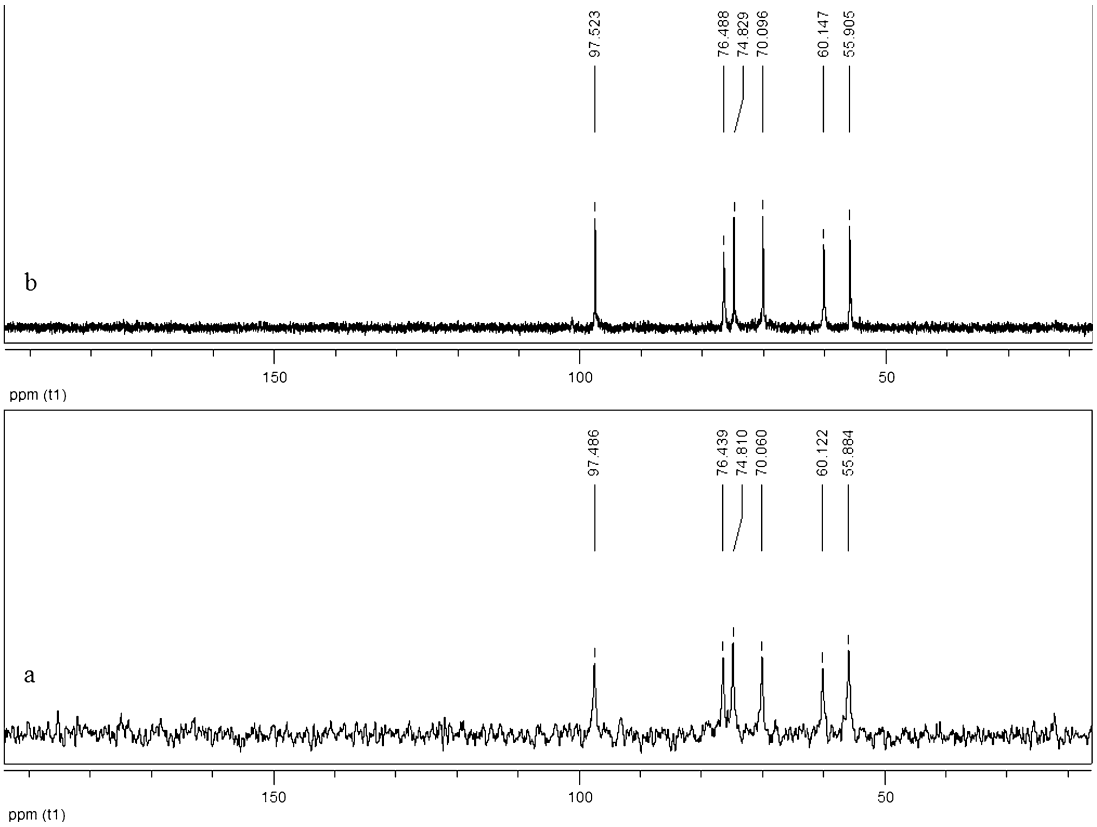

Fig. 5. ^{13}C NMR spectra. (a) Reference chitosan A; (b) irradiated chitosan A.

oxygen with doses of 25 kGy. The DD of irradiated chitosan was determined after the irradiation.

It was found that the DD of irradiated chitosan did not apparently change when the pH value ranged from 1.0 to 3.0, suggesting that the protonated amino groups of irradiated chitosan are stable enough to the strong oxidization resulting from gamma irradiation in the presence of oxygen. When pH value increased from 3.0 to 5.0, the DD of irradiated chitosan slightly decreased, compared with the reference chitosan.

3.6. *Effect of the irradiation in the presence of oxygen on chemical structure of irradiated chitosan*

Reference chitosan A solution (1%, w/v, pH 1.0, and 400 ml) under oxygen pressure of 201.325 kPa was irradiated with doses of 25 kGy. The irradiated chitosan with Mw of 10.4 kDa was obtained after the irradiation. To investigate its chemical structure, the irradiated chitosan was analyzed by FT-IR, ^{13}C NMR, and UV–vis spectroscopy, respectively.

For the FT-IR spectra of reference chitosan A as well as irradiated chitosan A, the broad peak around 3450 cm^{-1} are attributed to —NH_2 and O—H stretching. The characteristic absorption peaks appear at about 1648 (amide I band), 1598 (—NH_2 deformation mode) and 1324 cm^{-1} (amide III band). And the absorption peaks at 1160 (anti-symmetric stretching of the C—O—C bridge), 1078 and 1028 cm^{-1} (skeletal vibrations involving the C—O stretching) are characteristics of saccharide structure of chitosan. The small absorption peak at 896 cm^{-1} can be used to identify the presence of the C—O—C bridge as well as β-glucosidic linkages between the sugar units in chitosan. The assignments of absorption peaks are based on data reported in the literature (Kasaai, 2008; Pearson, Marchessault, & Liang, 1960; Zhou et al., 2008). For the spectrum of irradiated chitosan A, the amide I band had shifted to a lower wave number, it suggested that carbonyl groups had more opportunity to form stronger hydrogen bonds (Li, Cai, Zhong, & Du, 2012). It can be found that the irradiated chitosan A has the almost same absorption peaks as the reference chitosan A.

In the ^{13}C NMR spectra shown in Fig. 5, the irradiated chitosan A has the almost same signals as the reference chitosan A at about 55.905, 60.147, 70.096, 74.829, 76.488 and 97.523 ppm, which are attributed to C-2, C-6, C-3, C-5, C-4 and C-1, respectively.

It has been reported that depolymerization of polysaccharides by gamma irradiation led to characteristic absorption bands between 240 and 350 nm in UV–vis spectra because of some other chemical changes such as the formation of carbonyl, carboxyl, and double bonds according to Ulański and Rosiak (1992), Nagasawa et al. (2000) and Wasikiewicz et al. (2005). However, for the irradiation in the presence of oxygen, the irradiated chitosan A solution has no obvious characteristic absorption bands between 240 and 350 nm in UV–vis spectra shown in Fig. 6. It is further proved that

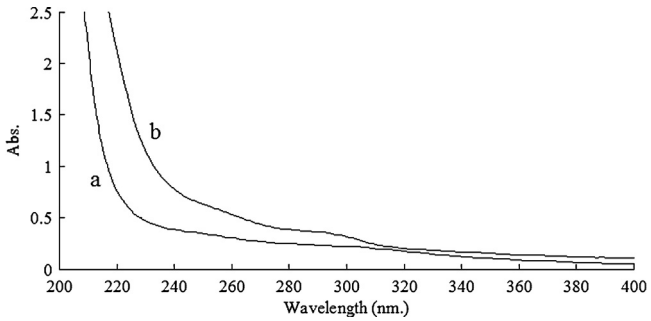

Fig. 6. UV–vis spectra. (a) Reference chitosan A solution (1%, w/v); (b) irradiated chitosan A solution (1%, w/v).

irradiation of chitosan in aqeous solution in the presence of sufficient oxygen is entirely different from that in the absence of oxygen.

The FT-IR, ^{13}C NMR, and UV–vis spectra suggested that the irradiation in the presence of sufficient oxygen could not result in modification of chemical structure of irradiated chitosan during depolymerization of reference chitosan and that the depolymerization reaction was mainly the cleavage of β-glycosidic linkages.

4. Conclusion

Oxygen and pH value could play important roles in the inhibiting browning of chitosan exposed to gamma radiation. When the pH value of chitosan solution was kept below 3.0, the sufficient oxygen could inhibit browning of irradiated chitosan in aqueous solution. The irradiated chitosan solutions were entirely colorless and pellucid as a result of the irradiation in the presence of oxygen. The DD of irradiated chitosan did not apparently change when the pH value ranged from 1.0 to 3.0. FT-IR, ^{13}C NMR, and UV–vis spectra confirmed that the irradiation in the presence of oxygen could not result in modification of chemical structure of irradiated chitosan. An effective technology can be developed for the inhibition of browning of irradiated chitosan during depolymerization of chitosan by gamma irradiation. The inhibition of browning of irradiated chitosan is promisingly useful for scale-up industrial manufacture of low-molecular-weight chitosan or chitooligosaccharides by gamma irradiation.

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References

Casimiro, M. H., Gil, M. H., & Leal, J. P. (2010). Suitability of gamma irradiated chitosan based membranes as matrix in drug release system. *International Journal of Pharmaceutics*, *395*(1–2), 142–146.

Choi, W.-S., Ahn, K.-J., Lee, D.-W., Byun, M.-W., & Park, H.-J. (2002). Preparation of chitosan oligomers by irradiation. *Polymer Degradation and Stability*, *78*(3), 533–538.

Dong, Y., Wang, H., Zheng, W., Zhao, Y., Bi, D., Zhao, L., et al. (2004). Liquid crystalline behaviour of chitooligosaccharides. *Carbohydrate Polymers*, *57*(3), 235–240.

Dou, J., Ma, P., Xiong, C., Tan, C., & Du, Y. (2011). Induction of apoptosis in human acute leukemia HL-60 cells by oligochitosan through extrinsic and intrinsic pathway. *Carbohydrate Polymers*, *86*(1), 19–24.

Duy, N. N., Phu, D. V., Anh, N. T., & Hien, N. Q. (2011). Synergistic degradation to prepare oligochitosan by γ-irradiation of chitosan solution in the presence of hydrogen peroxide. *Radiation Physics and Chemistry*, *80*(7), 848–853.

Feng, T., Du, Y., Li, J., Hu, Y., & Kennedy, J. F. (2008). Enhancement of antioxidant activity of chitosan by irradiation. *Carbohydrate Polymers*, *73*(1), 126–132.

Guo, Z., Ren, J., Dong, F., Wang, G., & Li, P. (2013). Comparative study of the influence of active groups of chitosan derivatives on antifungal activity. *Journal of Applied Polymer Science*, *127*(4), 2553–2556.

Hai, L., Diep, T. B., Nagasawa, N., Yoshii, F., & Kume, T. (2003). Radiation depolymerization of chitosan to prepare oligomers. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, *208*, 466–470.

Hien, N. Q., Phu, D. V., Duy, N. N., & Lan, N. T. K. (2012). Degradation of chitosan in solution by gamma irradiation in the presence of hydrogen peroxide. *Carbohydrate Polymers*, *87*(1), 935–938.

Hodge, J. E. (1953). Dehydrated foods, chemistry of browning reactions in model systems. *Journal of Agricultural and Food Chemistry*, *1*(15), 928–943.

Hu, Y., Du, Y., Yang, J., Kennedy, J. F., Wang, X., & Wang, L. (2007). Synthesis, characterization and antibacterial activity of guanidinylated chitosan. *Carbohydrate Polymers*, *67*(1), 66–72.

Je, J.-Y., Park, P.-J., & Kim, S.-K. (2004). Radical scavenging activity of heterochitooligosaccharides. *European Food Research and Technology*, *219*(1), 60–65.

Kang, B., Dai, Y. D., Zhang, H. Q., & Chen, D. (2007). Synergetic degradation of chitosan with gamma radiation and hydrogen peroxide. *Polymer Degradation and Stability*, *92*(3), 359–362.

Kasaai, M. R. (2008). A review of several reported procedures to determine the degree of N-acetylation for chitin and chitosan using infrared spectroscopy. *Carbohydrate Polymers*, *71*(4), 497–508.

Katsuno, S., Shimamura, T., Kashiwagi, T., Izawa, N., & Ukeda, H. (2013). Effects of dissolved oxygen on the Maillard reaction during heat treatment of milk. *International Dairy Journal*, *33*(1), 34–37.

Kim, S.-K., & Rajapakse, N. (2005). Enzymatic production and biological activities of chitosan oligosaccharides (COS): A review. *Carbohydrate Polymers*, *62*(4), 357–368.

Kong, M., Chen, X. G., Xing, K., & Park, H. J. (2010). Antimicrobial properties of chitosan and mode of action: A state of the art review. *International Journal of Food Microbiology*, *144*(1), 51–63.

Li, J., Cai, J., Zhong, L., & Du, Y. (2012). Immobilization of a protease on modified chitosan beads for the depolymerization of chitosan. *Carbohydrate Polymers*, *87*(4), 2697–2705.

Li, Y., Wang, Y., Wu, D., Zhang, K., & Hu, Q. (2010). A facile approach to construct three-dimensional oriented chitosan scaffolds with in-situ precipitation method. *Carbohydrate Polymers*, *80*(2), 408–412.

Litchfield, J. E., Thorpe, S. R., & Baynes, J. W. (1999). Oxygen is not required for the browning and crosslinking of protein by pentoses: Relevance to Maillard reactions in vivo. *International Journal of Biochemistry & Cell Biology*, *31*(11), 1297–1305.

Martins, S. I. F. S., Jongen, W. M. F., & Boekel, M. A. J. S. V. (2000). A review of Maillard reaction in food and implications to kinetic modeling. *Trends in Food Science & Technology*, *11*(9–10), 364–373.

Matsuhashi, S., & Kume, T. (1997). Enhancement of antimicrobial activity of chitosan by irradiation. *Journal of the Science of Food and Agriculture*, *73*(2), 237–241.

Muzzarelli, R. A. A. (2011). Potential of chitin/chitosan-bearing materials for uranium recovery: An interdisciplinary review. *Carbohydrate Polymers*, *84*(1), 54–63.

Muzzarelli, R. A. A., Boudrant, J., Meyer, D., Manno, N., DeMarchis, M., & Paoletti, M. G. (2012). Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: A tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial. *Carbohydrate Polymers*, *87*(2), 995–1012.

Muzzarelli, R. A. A., Greco, F., Busilacchi, A., Sollazzo, V., & Gigante, A. (2012). Chitosan, hyaluronan and chondroitin sulfate in tissue engineering for cartilage regeneration: A review. *Carbohydrate Polymers*, *89*(3), 723–739.

Muzzarelli, R. A. A., & Tubertini, O. (1972). Radiation resistance of chitin and chitosan applied in the chromatography of radioactive solutions. *Journal of Radioanalytical Chemistry*, *12*(1), 431–440.

Nagasawa, N., Mitomo, H., Yoshii, F., & Kume, T. (2000). Radiation-induced degradation of sodium alginate. *Polymer Degradation and Stability*, *69*(3), 279–285.

Ocloo, F. C. K., Quayson, E. T., Adu-Gyamfi, A., Quarcoo, E. A., Asare, D., Serfor-Armah, Y., et al. (2011). Physicochemical and functional characteristics of radiation-processed shrimp chitosan. *Radiation Physics and Chemistry*, *80*(7), 837–841.

Oh, S. H., Lee, Y. S., Kim, J. H., Kim, J. H., Lee, J. W., Kim, M. R., et al. (2006). Effect of pH on non-enzymatic browning reaction during γ-irradiation processing using sugar and sugar–glycine solutions. *Food Chemistry*, *94*(3), 420–427.

Pasanphan, W., Rimdusit, P., Choofong, S., Piroonpan, T., & Nilsuwankosit, S. (2010). Systematic fabrication of chitosan nanoparticle by gamma irradiation. *Radiation Physics and Chemistry*, *79*(10), 1095–1102.

Pearson, F. G., Marchessault, R. H., & Liang, C. Y. (1960). Infrared spectra of crystalline polysaccharides. V. Chitin. *Journal of Polymer Science*, *43*(141), 101–116.

Rahman, M. M., Kabir, S., Rashid, T. U., Nesa, B., Nasrin, R., Haque, P., et al. (2013). Effect of γ-irradiation on the thermomechanical and morphological properties of chitosan obtained from prawn shell: Evaluation of potential for irradiated chitosan as plant growth stimulator for Malabar spinach. *Radiation Physics and Chemistry*, *82*, 112–118.

Rao, M. S., Chawla, S. P., Chander, R., & Sharma, A. (2011). Antioxidant potential of Maillard reaction products formed by irradiation of chitosan–glucose solution. *Carbohydrate Polymers*, *83*(2), 714–719.

Sashiwa, H., & Aiba, S. (2004). Chemically modified chitin and chitosan as biomaterials. *Progress in Polymer Science*, *29*(9), 887–908.

Shen, K., Hu, Q., Wang, Z., & Qu, J. (2011). Effect of ^{60}Co irradiation on the properties of chitosan rod. *Materials Science and Engineering C*, *31*(5), 866–872.

Shi, X.-Y., & Tan, T.-W. (2002). Preparation of chitosan/ethylcellulose complex microcapsule and its application in controlled release of Vitamin D_2. *Biomaterials*, *23*(23), 4469–4473.

Singh, D., & Singh, R. (2012). Papain incorporated chitin dressings for wound debridement sterilized by gamma radiation. *Radiation Physics and Chemistry*, *81*(11), 1781–1785.

Tang, Y., Wang, X., Li, Y., Lei, M., Du, Y., Kennedy, J. F., et al. (2010). Production and characterisation of novel injectable chitosan/methylcellulose/salt blend hydrogels with potential application as tissue engineering scaffolds. *Carbohydrate Polymers*, *82*(3), 833–841.

Tolaimate, A., Desbrieres, J., Rhazi, M., Alagui, A., Vincendon, M., & Vottero, P. (2000). On the influence of deacetylation process on the physicochemical characteristics of chitosan from squid chitin. *Polymer*, *41*(7), 2463–2469.

Ulański, P., & Rosiak, J. (1992). Preliminary studies on radiation-induced changes in chitosan. *International Journal of Radiation Applications and Instrumentation. Part C: Radiation Physics and Chemistry*, *39*(1), 53–57.

Wasikiewicz, J. M., Yoshii, F., Nagasawa, N., Wach, R. A., & Mitomo, H. (2005). Degradation of chitosan and sodium alginate by gamma radiation, sonochemical

and ultraviolet methods. *Radiation Physics and Chemistry*, *73*(5), 287–295.

Wu, H., Yao, Z., Bai, X., Du, Y., & Ma, X. (2010). Chitooligosaccharides inhibit nitric oxide mediated migration of endothelial cells in vitro and tumor angiogenesis in vivo. *Carbohydrate Polymers*, *82*(3), 927–932.

Xia, W., Liu, P., Zhang, J., & Chen, J. (2011). Biological activities of chitosan and chitooligosaccharides. *Food Hydrocolloids*, *25*(2), 170–179.

Xu, J., Zhao, X., Han, X., & Du, Y. (2007). Antifungal activity of oligochitosan against *Phytophthora capsici* and other plant pathogenic fungi *in vitro*. *Pesticide Biochemistry and Physiology*, *87*(3), 220–228.

Xu, Q., Dou, J., Wei, P., Tan, C., Yun, X., Wu, Y., et al. (2008). Chitooligosaccharides induce apoptosis of human hepatocellular carcinoma cells via up-regulation of Bax. *Carbohydrate Polymers*, *71*(4), 509–514.

Yin, H., Zhao, X., & Du, Y. (2010). Oligochitosan: A plant diseases vaccine—A review. *Carbohydrate Polymers*, *82*(1), 1–8.

Yoksan, R., Akashi, M., Miyata, M., & Chirachanchai, S. (2004). Optimal γ-ray dose and irradiation conditions for producing low-molecular-weight chitosan that retains its chemical structure. *Radiation Research*, *161*(4), 471–480.

Zeng, L., Qin, C., Chi, W., Wang, L., Ku, Z., & Li, W. (2007). Browning of chitooligomers and their optimum preservation. *Carbohydrate Polymers*, *67*(4), 551–558.

Zhang, K., Zhao, M., Cai, L., Wang, Z., Sun, Y., & Hu, Q. (2010). Preparation of chitosan/hydroxyapatite guided membrane used for periodontal tissue regeneration. *Chinese Journal of Polymer Science*, *28*(4), 555–561.

Zhao, M., Li, L., Li, X., Zhou, C., & Li, B. (2011). Three-dimensional honeycomb-patterned chitosan/poly(L-lactic acid) scaffolds with improved mechanical and cell compatibility. *Journal of Biomedical Materials Research Part A*, *98A*(3), 434–441.

Zhou, H. Y., Chen, X. G., Kong, M., Liu, C. S., Cha, D. S., & Kennedy, J. F. (2008). Effect of molecular weight and degree of chitosan deacetylation on the preparation and characteristics of chitosan thermosensitive hydrogel as a delivery system. *Carbohydrate Polymers*, *73*(2), 265–273.

Zhou, H. Y., Zhang, Y. P., Zhang, W. F., & Chen, X. G. (2011). Biocompatibility and characteristics of injectable chitosan-based thermosensitive hydrogel for drug delivery. *Carbohydrate Polymers*, *83*(4), 1643–1651.